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Short Communication

Diethanolamine Cytotoxicity on Red Blood Corpuscles

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Abstract

The purpose of this study was to evaluate the effect of diethanolamine (DEA) on red blood corpuscles (RBC) in *in vitro* condition*. Intravenous blood samples from healthy adult volunteers (age 25-30 years) were collected in EDTA vials and used for preparation of RBC suspension in saline (0.9% NaCl). RBC suspension was incubated with different concentrations (100-1000 µg/ml) of diethanolamine at 37 ºC for 4 h. Morphological alterations and percent hemolysis were recorded. Statistical analysis was performed using the analysis of variance (ANOVA) followed by Tukey's test and the level of significance was accepted with * p<0.05. The results showed that diethanolamine caused concentration-dependent significant increase (P<0.05) in hemolysis.*

Keywords: Diethanolamine (DEA), red blood corpuscles, hemolysis, cytotoxicity.

Introduction

Exposure to toxic chemicals are connected to some of our country's most serious health problems. Humans beings are unwittingly exposed to numerous harmful chemicals. These chemicals can find their way into the body and are capable of causing harm. Diethanolamine (DEA) is an alkanolamine which is widely used as industrial chemicals[1], metal working fluids, agriculture chemicals and consumer products like cosmetics, soaps and shampoos[2,3]. It is used in pharmaceutical industries as buffer and stabilizer for certain drugs[4] and also used as raw materials in the production of some drugs. General population may be exposed to DEA through cigarette smoking[5], consumer products (cosmetics, soaps and shampoos) via dermal exposure and occupational exposure by inhalation of vapors and aerosols and by skin contact during the use of DEA in many industries[6,7]. According to National Occupational Exposure Survey as many as 800,000 workers get exposed to DEA per year[8]. DEA is readily absorbed through skin. NTP reported that exposure to DEA caused toxic effect on liver, kidney and testis by oral and dermal exposure[9]. It has been previously reported DEA caused tumor in liver and kidney of mice by dermal exposure[10]. National Toxicology Program also reported that DEA caused microcytic anemia, decrease in erythrocyte, reticulocyte counts, hemoglobin concentration and hematocrit in their oral and dermal study[9]. However, no data are available to the effect of DEA on RBC. Therefore, we undertook the present investigation to evaluate the effect of DEA on human red blood corpuscles in *in vitro* condition.

Material and Methods

Diethanolamine was purchased from Merck specialities Pvt. Ltd., Mumbai, India. All other chemicals used were of analytical grade.

Preparation of RBC suspension: Intravenous blood samples were collected from healthy well nourished volunteers of 25-30 years of age in EDTA vials. Blood samples were diluted with normal saline (0.9 % NaCl) and centrifuged at 1000×g for 10 min. RBC pellets were washed twice and finally diluted with saline to get cell density of $2x10^4$ cells/Ml[11,12]. For this experiment diethanolamine was also prepared in normal saline (0.9% NaCl).

Study design: To evaluate the toxic effect of diethanolamine on RBC, following sets of tubes were prepared. i. Control tubes containing 2 mL of RBC suspension and 2 mL of saline. ii. DEA treated tubes containing 2 mL of RBC suspension and 100 to 1000 µg/mL DEA. The total volume of each tube was made to 4 mL with addition of saline. iii. 100% hemolysis tubes containing 2 mL of distilled water and 2 mL of RBC suspension.

The incubation medium containing RBC suspension and DEA in saline were mixed gently and incubated at 37ºC for 4 h with intermittent shaking. Thereafter the tubes were centrifuged at 1000×g for 10 min. The colour density of supernatant was measured spectrophotometrically at 540 nm. Morphological changes were also observed in RBC.

Calculation: Hemolysis (%) was calculated by the formula.

$$\% \text{ hemolysis} = \frac{\text{Absorbance of individual tubes}}{\text{Absorbance with 100\% hemolysis}} \times 100$$

Statistical Analysis: Statistical analysis was performed by analysis of variance (ANOVA) followed by Tukey's test using GraphPad prism software. Data is expressed as the means ± S.E.M. Accepted significance level was *p<0.05. Pearson's

correlation analysis was used to determine the correlation between control and treated tubes.

Results and Discussion

Diethanolamine cytotoxicity was examined in human red blood corpuscles. In control tubes the supernatant remained clear and RBC setteled in the bottom of the tubes were normal. In DEA treated tubes reddish colour appeared in supernatant which indicates hemolysis. In DEA treated tubes number of cells settled in the bottom of the tubes reduced. Diethanolamine treated RBC showed swelling. Addition of DEA (100-1000µg/ml) to suspension of RBC caused significant ($p<0.05$) increase in hemolysis (figure 1). This increase was concentration-dependent ($r=0.9065$). Hemolysis was maximum on addition of 900 µg/ml of DEA Hemolysis may be because of influx of DEA into the cells causing alteration in RBC membrane, swelling and eventual cell lysis. However, the exact mechanism of action is not clearly understood. DEA disturbs phospholipids metabolism, structure and function[9,13]. RBC membrane contains 60% phospholipids of the total lipid components. Lipid composition is important for membrane permeability and fluidity. DEA is known to create choline deficiency. Choline is essential nutrient for proper cell growth and function. It has been previously reported that DEA competitively inhibits the cellular uptake of choline *in vitro*[14,15]. Choline deficiency include increased generation of free radicals and increased susceptibility to oxidative damage[16]. Thus hemolysis might be due to oxidative damage and alteration of phospholipid membrane.

Conclusion

The present study clearly indicates that DEA is cytotoxic which caused concentration-dependent increase in hemolysis. This hemolysis may be due to alteration in membrane and oxidative damage.

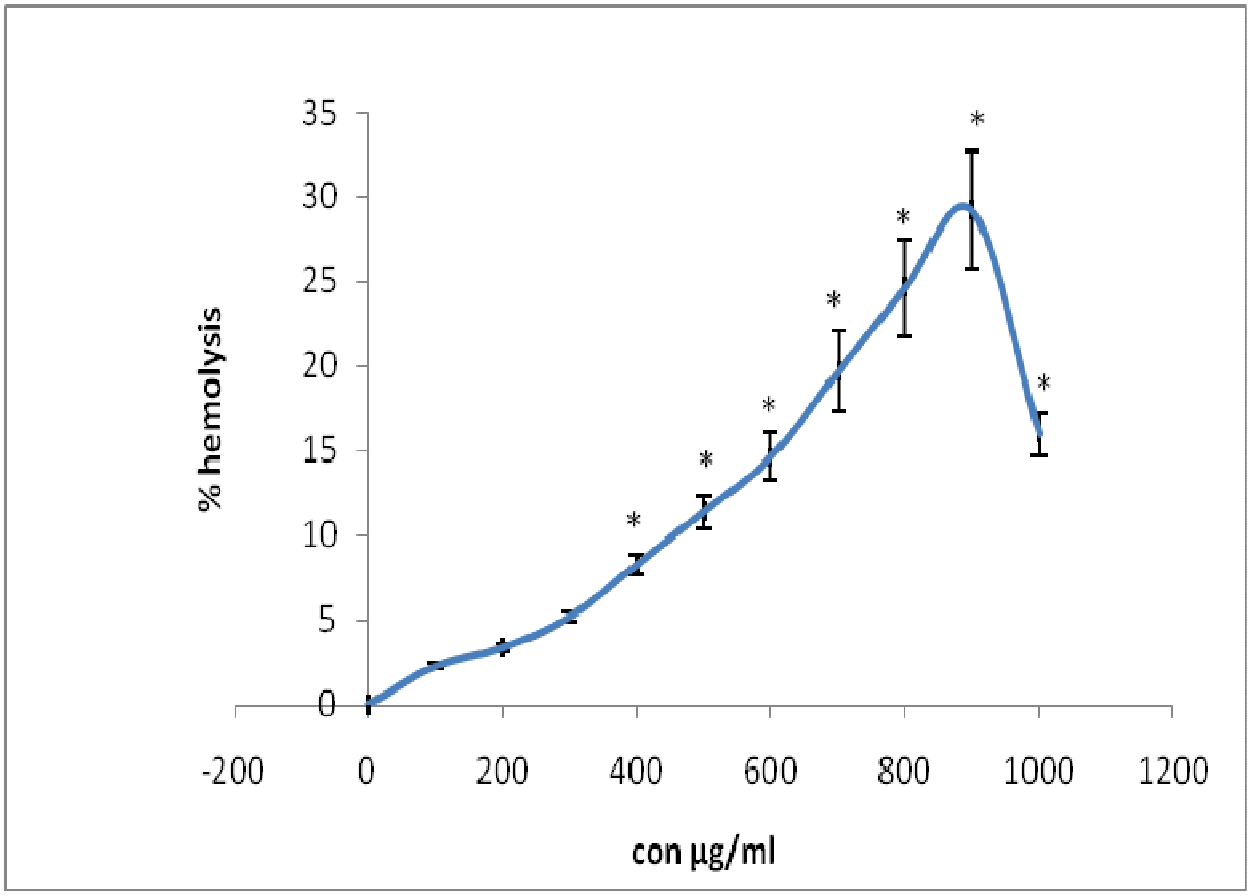

Figure-1
Showing effect of diethanolamine on rate of hemolysis. The values are mean±S.E.M; n=10. Significant at the level $p<0.05$ as compared to control

Reference

1. Wagner P., Reassessment of Diethanolamine. (CAS Reg. No.111-42-2) United States Environmental Protection Agency, Washington, D.C. 20460.July 31 **(2006)**

2. CIR Cosmetic Ingredients Review, Final report on the safety assessment of triethanolamine, diethanolamine, and monoethanolamine, *J Am Coll Toxicol*, **2**, 183–235 **(1983)**

3. CIR Cosmetic Ingredients Review. Final report on the safety assessment of cocamide DEA, lauramide DEA, linoleamiede DEA, and oleamide DEA, *J Am Coll Toxicol*, **5**, 415–454 **(1986)**

4. Soreat S A., Stabilizing acetylsalicylic acid and its salts in solution. Fr. Demande. **2,** 143, 609 **(1973)**.

5. Hoffmann D., Brunnemann K.D., Rivenson A. and Hecht S.S., N nitrosiodiethanolamine: analysis, formation in tobacco products and carcinogenicity in Syrian golden hamsters, *IARC Sci Publ*, **41**, 299-308 **(1982)**

6. Knaak J.B., Leung H.W., Stott W.T., Busch J. and Bilsky J., Toxicology of mono-di- and triethanolamine, *Rev Environ Contam Toxicol*, **7**, 149-186 **(1997)**

7. Blum A. and Lischka G., Allergic contact dermatitis from monoethanolamine, diethanolamine and triethanolamine, *Contact dermatitis*, **36(3),** 166 **(1997)**

8. Technology Planning and Management Corporation (TPMC). Report on carcinogens Background Document for Diethanolamine, In National Toxicology Program NIEHS, Durham, NC. NO1ES85421, 229 **(2002)**

9. National Toxicological Programm {NTP}. NTP technical report on the toxicity Studies of Diethanolamine (CAS No 111-42-2) administered topically and in drinking water to F344/ N Rats and B6C3F1 mice, Toxic Report Series ;20:1-D10 **(1992)**

10. Toxicology and carcinogenesis studies of diethanolamine," National Toxicology Program (NTP), U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NTP TR 478 (NIH Publication No. 99-3968), pages 1–22 July **(1999)**

11. Verma R J, Sangai N P., The ameliorative effect of black tea extract and

quercetin on bisphenol A induced cytotoxicity. Acta Poloniae Pharmaceutica Drug Research, **66,** 41-44 **(2009)**.

12. Verma R J, Raval P J., Cytotoxicity of aflatoxin on red blood corpuscles. Bulletin of Environmental Contamination and Toxicology, **17**, 457 **(2003)**.

13. Methews JM, Garner CE and Matthews HB., Metabolism, bioaccumulation and incorporation of diethanolamine into phospholipids, *Chemical Research Toxicology*, **8**, 625-633 **(1995).**

14. Lehman- McKeeman LD and Gamsky EA, Diethanolamine inhibits choline uptake and phosphatidylcholine synthesis in Chinese hamster ovary cells. Biochemical Biophysics Research Communication, **262**, 600-604 **(1999)**.

15. Lehman- McKeeman LD and Gamsky EA., Choline supplementation inhibits Diethanolamine induced morphological transformation in Syrian hamster embryo cells: Evidence for a carcinogenic mechanism Toxicological Sciences, **55**, 303-310 **(2000)**.

16. Floyd RA , Kotake Y, Hensley K, Nakae D and Konishi Y., Reactive oxygen species in choline deficiency induced carcinogenesis and nitrone inhibition, *Mol Cell Biochem*, 234-235 **(1-2),** 195-203 **(2002)**